

1 **Claims**

2

3 1. A method of producing an oligopeptide product,
4 the method comprising the steps:

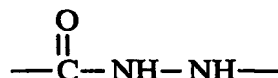
5 a) providing a first oligopeptide, the first
6 oligopeptide having a reactive moiety,

7 b) providing a second oligopeptide, the second
8 oligopeptide having a activated ester moiety

9 c) allowing the reactive moiety of the first
10 oligopeptide to react with the activated ester
11 moiety of the second oligopeptide to form an
12 oligopeptide product, in which the first and second
13 oligopeptides are linked via a linking moiety having
14 Formula I, Formula II or Formula III.

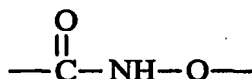
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16 Formula I



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18 Formula II



19

20 Formula III



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24 2. The method according to claim 1 wherein the
25 terminal activated ester moiety is a thioester
26 wherein the peptide is the acyl substituent of

1 the thioester.

2

3 3. The method according to claim 2, wherein said
4 second polypeptide is generated by thiol reagent
5 dependent cleavage of a precursor molecule, said
6 precursor molecule comprising a second oligopeptide
7 fused N-terminally to an intein domain.

8

9 4. A method of producing an oligopeptide product,
10 the method comprising the steps:
11 a) providing a first oligopeptide, the first
12 oligopeptide having a reactive moiety,
13 (i) providing a precursor oligopeptide molecule, the
14 precursor oligopeptide molecule comprising a second
15 oligopeptide fused N-terminally to an intein domain
16 (ii) allowing thiol reagent dependent cleavage of
17 the precursor molecule to generate a second
18 oligopeptide molecule, said second oligopeptide
19 molecule having a thioester moiety at its C-
20 terminus,
21 c) allowing the reactive moiety of the first
22 oligopeptide to react with the second oligopeptide
23 molecule to form an oligopeptide product, in which
24 the first and second oligopeptides are linked via a
25 linking moiety having Formula I, II or III.

26

27 5. The method according to any one of the preceding
28 claims wherein the reactive moiety is a hydrazine
29 moiety, a hydrazide moiety or an aminooxy moiety.

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31 6. The method according to claim 5, wherein the
32 reactive moiety is an aminooxy moiety and the

1 activated ester moiety is a thioester.

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3 7. The method according to claim 5, wherein said
4 first oligopeptide is produced by reaction of
5 hydrazine with a precursor molecule, said
6 precursor molecule comprising a precursor
7 oligopeptide fused N-terminally to an intein
8 domain via a thioester moiety.

9

10 8. A method of producing an oligopeptide product,
11 said method comprising the steps:
12 a) providing a first oligopeptide, the first
13 oligopeptide having a reactive moiety, wherein
14 the reactive moiety is a hydrazine moiety, a
15 hydrazide moiety or an amino-oxy moiety;
16 (i) providing a precursor oligopeptide molecule,
17 the precursor oligopeptide molecule comprising a
18 second oligopeptide fused N-terminally to an
19 intein domain;
20 (c) allowing the reactive moiety of the first
21 oligopeptide to react with the precursor
22 oligopeptide molecule to form an oligopeptide
23 product, in which the first and second
24 oligopeptides are linked via a linking moiety
25 having Formula I, Formula II or Formula III.

26

27 9. The method according to any one of the preceding
28 claims, wherein the first oligopeptide or the
29 second oligopeptide is a recombinant oligopeptide
30 and the other of the the first oligopeptide and
31 the second oligopeptide is a synthetic
32 polypeptide.

1

2 10. The method according to any one of claims 1 to
3 8, wherein the first oligopeptide and the second
4 oligopeptide are recombinant oligopeptides.

5

6 11. The method according to any one of claims 1 to
7 8, wherein the first oligopeptide and the second
8 oligopeptide are synthetic oligopeptides.

9

10 12. A method of generating a protein hydrazide,
11 said method comprising the steps:
12 (a) providing a protein molecule comprising an
13 oligopeptide fused N-terminal to an intein
14 domain,
15 (b) reacting said protein molecule with
16 hydrazine, such that the intein domain is cleaved
17 from the oligopeptide to generate a protein
18 hydrazide.

19

20 13. The method according to any one of the claims 1
21 to 11 wherein step (c) of the method is performed
22 at a pH in the range pH 6.5 to 7.5.

23

24 14. A method of producing an oligopeptide product,
25 the method comprising the steps:
26 a) providing a first oligopeptide, the first
27 oligopeptide having an aldehyde or ketone moiety,
28 b) providing a precursor oligopeptide molecule,
29 the precursor oligopeptide molecule comprising a
30 second oligopeptide fused N-terminally to an
31 intein domain,
32 c) reacting said precursor oligopeptide molecule

1 with hydrazine to generate an oligopeptide
2 molecule comprising an intermediate oligopeptide,
3 said intermediate oligopeptide having a terminal
4 hydrazide moiety,
5 d) allowing the aldehyde or ketone moiety of the
6 first oligopeptide to react with the hydrazide
7 moiety of the intermediate oligopeptide molecule
8 to form an oligopeptide product, in which first
9 oligopeptide and the second oligopeptide are
10 linked via a hydrazone linking moiety.

11

12 15. An oligopeptide product produced by the method
13 of any one of the preceding claims.

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15 16. A method of labelling an oligopeptide, the
16 method comprising the steps:
17 a) providing a label molecule, the label molecule
18 having a reactive moiety,
19 b) providing the oligopeptide, the oligopeptide
20 having a activated ester moiety
21 c) allowing the reactive moiety of the label
22 molecule to react with the activated ester moiety
23 of the oligopeptide to form the labelled
24 oligopeptide, in which the label molecule and the
25 oligopeptide are linked via a linking moiety
26 having Formula I, Formula II or Formula III.

27

28 17. The method according to claim 16, wherein in
29 step (c), where said label molecule and the
30 oligopeptide are linked via a linking moiety
31 having Formula II and where said activated ester
32 moiety of step (b) is not a thioester, said

1 activated ester is a terminal activated ester
2 moiety.

3

4 18. A method of labelling an oligopeptide, the
5 method comprising the steps:
6 a) providing a label molecule, the label molecule
7 having an activated ester moiety of which the
8 label is the acyl substituent,
9 b) providing the oligopeptide, the oligopeptide
10 having a reactive moiety
11 c) allowing the activated ester moiety of the
12 label molecule to react with the reactive moiety
13 of the oligopeptide to form the labelled
14 oligopeptide, in which the label molecule and the
15 oligopeptide are linked via a linking moiety
16 having Formula I, Formula II or Formula III,
17 wherein, in step (c), where said label molecule
18 and the oligopeptide are linked via a linking
19 moiety having Formula II and where said activated
20 ester moiety of step (b) is not a thioester, said
21 activated ester is a terminal activated ester
22 moiety.

23

24 19. The method according to claim 18 wherein said
25 oligopeptide is produced by reaction of hydrazine
26 with a precursor molecule, said precursor
27 molecule comprising a precursor oligopeptide
28 fused N-terminally to an intein domain via a
29 thioester moiety.

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31 20. A method of labelling an oligopeptide, the
32 method comprising the steps:

- 1 a) providing a label, the label having a reactive
2 moiety,
3 (i) providing a precursor oligopeptide molecule,
4 the precursor oligopeptide molecule comprising an
5 oligopeptide fused N-terminally to an intein
6 domain
7 (ii) allowing thiol reagent dependent cleavage of
8 the precursor molecule to generate the
9 oligopeptide molecule, said oligopeptide molecule
10 having a thioester moiety at its C-terminus,
11 c) allowing the reactive moiety of the label to
12 react with the oligopeptide molecule to form a
13 labelled oligopeptide, in which the label and
14 oligopeptide are linked via a linking moiety
15 having Formula I, II or III.
16
- 17 21. The method according to any one of claims 16 to
18 18, wherein the reactive moiety is an aminooxy
19 moiety and the activated ester moiety is a
20 thioester.
21
- 22 22. The method according to claim 20, wherein the
23 reactive moiety is an aminooxy moiety.
24
- 25 23. A method of labelling an oligopeptide, the
26 method comprising the steps:
27 a) providing a label molecule, the label molecule
28 having a reactive moiety,
29 b) providing a precursor oligopeptide molecule,
30 the precursor oligopeptide molecule comprising an
31 oligopeptide fused N-terminally to an intein
32 domain,

1 c) allowing the reactive moiety of the label
2 molecule to react with the precursor oligopeptide
3 molecule to form a labelled oligopeptide product,
4 in which the label molecule and the oligopeptide
5 are linked via a linking moiety having Formula I,
6 Formula II or Formula III, as defined above.

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8 24. The method according to any one of claims 16 to
9 23 wherein step (c) of the method is performed at
10 a pH in the range pH 6.5 to pH 7.5.

11

12 25. A method of labelling an oligopeptide, the
13 method comprising the steps:
14 a) providing a label molecule, the label molecule
15 having a aldehyde or ketone moiety,
16 b) providing a precursor oligopeptide molecule,
17 the precursor oligopeptide molecule comprising a
18 first oligopeptide fused N-terminally to an
19 intein domain,
20 c) reacting said precursor oligopeptide molecule
21 with hydrazine to generate an oligopeptide
22 molecule comprising an intermediate oligopeptide,
23 said intermediate oligopeptide having a terminal
24 hydrazide moiety,
25 d) allowing the aldehyde or ketone moiety of the
26 label molecule to react with the hydrazide moiety
27 of the intermediate oligopeptide molecule to form
28 a labelled oligopeptide product, in which the
29 label molecule and oligopeptide are linked via a
30 hydrazone linking moiety.

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1 26. The method according to claim 14 or claim 25,
2 wherein the aldehyde or ketone moiety is an α -
3 diketone or an α -keto-aldehyde group.

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5 27. A labelled oligopeptide produced by the method
6 of any one of claims 16 to 26.

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